Video Article

Deciphering Axonal Pathways of Genetically Defined Groups of Neurons in the Chick Neural Tube Utilizing *in ovo* Electroporation

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Abstract

Employment of enhancer elements to drive expression of reporter genes in neurons is a widely used paradigm for tracking axonal projection. For tracking axonal projection of spinal interneurons in vertebrates, germ line-targeted reporter genes yield bilaterally symmetric labeling. Therefore, it is hard to distinguish between the ipsi- and contra-laterally projecting axons. Unilateral electroporation into the chick neural tube provides a useful means to restrict expression of a reporter gene to one side of the central nervous system, and to follow axonal projection on both sides [1,2-5]. This video demonstrates first how to handle the eggs prior to injection. At HH stage 18-20, DNA is injected into the sacral level of the neural tube, then tungsten electrodes are placed parallel to the embryo and short electrical pulses are administered with a pulse generator. The egg is sealed with tape and placed back into an incubator for further development. Three days later (E6) the spinal cord is removed as an open book preparation from embryo, fixed, and processed for whole mount antibody staining. The stained spinal cord is mounted on slide and visualized using confocal microscopy.

Protocol

I. Electroporation

Egg handling

1. Place the eggs in an humidified incubator set to 37-38°C, preferable an incubator with a rocking trays.
2. Embryo are electroporated after about 66 hours of the incubation, when they have reached Hamburger & Hamilton (HH) stage 18-20. At this stage the head lies at right angles to the trunk (condition called cervical flexure) and a vast set of extra-embryonic blood vessels, such as anterior, posterior, right and left vitelline veins should be seen. This is an optimal stage for enhancer-dependent specific expression [1,3,4]. It is important to monitor temperature and humidity of the incubator, in order to obtain viable and synchronized embryos.
3. After 66 hours of incubation, remove the eggs from the incubator. Place the eggs horizontally. Wait 5-10 minutes. The embryo now is located at the upper pole of the egg.

Preparations

1. Prepare Hank's solution with penicillin/streptomycin (P/S) (1:100 dilution).
2. Pull glass capillaries (0.5 mm diameter) to microcapillary.
3. Place the electrodes (tungsten) into micromanipulator and connect electrodes to the pulse generator (ECM 830).
4. Adjust the pulse generator with the following parameters: voltage 30v, number of pulses - 3, length of the pulse 50 ms.
5. Prepare a mouth pipette, a syringe needle and a tape.
6. Prepare a desired mixture of DNA (2-5µg/µl) and add a Fast Green dye.

Windowing and electroporation

1. Using a syringe, remove 5-6 ml of albumin from each egg.
2. With a small scissors open an oval window at the upper side of the egg.
3. Moist the embryo with 0.5-1ml Hank's + P/S solution.
4. Use a mouth pipette, to load the glass microcapillary with DNA mixture.
5. Place the embryo with its tail toward you. At this stage, embryo's spinal cord is clearly seen. Hence, no contrasting techniques are required. The spinal cord is sealed at both ends, the head and the tail, but if your microcapillary is thin enough, you will be able to puncture a small hole and penetrate the neural tube at a shallow angle. A microcapillary of right diameter should be loaded easily with DNA, penetrate the tube without damaging it and release the DNA with a regular exhalation.
6. Inject the DNA using a mouth pipette. The green dye should spread from the tip of the tail up to the vesicles of the developing brain.
7. Place the electrodes in parallel to the neural tube, as near as you can, without touching the tube itself and pulse. At the time of the pulse, the electrodes must be covered with liquid for allowing electrical conductivity. Usually, the amount of Hank's added at the begging is sufficient. If not add one drop of Hank's just prior to the pulse.
8. Carefully remove the electrodes and seal the window with a tape. It is important to seal the egg entirely to prevent drying of the embryo during the following incubation period.
9. Place the egg back in the incubator.

II. Spinal cord open-book preparation

Detachment of the spinal cord from the embryo

1. Remove the eletroporated embryo, at E6, from the egg and place it in a Petri dish coated with silicone containing PBS.
2. Cut away the membranes and stretch the embryo on his ventral side using pins to hold it.
3. Using a sharp tungsten microcapillary make a longitudinal incision along the roof plate, from the hindbrain down to the tail.
4. Make an additional two longitudinal incisions at both sides of the spinal cord, detaching the dorsal root ganglia (DRGs) away from the spinal cord.
5. Detach the floor plate from the tissue starting from the tail to the hindbrain, leaving the spinal cord intact.
6. Cut the spinal cord in a transverse section at the hindbrain using fine scissors and separate it from the body.

Fixation

1. Spread the isolated spinal cord in a new petri dish coated with silicone containing PBS, using pins to hold it, from the hindbrain to the tail producing a flat-mount preparation.
2. Pour the PBS from the dish and replace it with 4% paraformaldehyde in phosphate-buffered saline.
3. Incubate at room temperature for 1 hour.

Immunohistochemistry

1. Transfer the fixed spinal cord into a vial containing primary antibody in PBS.
2. Incubate with gentle agitations over night at 4°C.
3. Wash the antibody X5 times with PBS, each wash for 1 hour with gentle agitations.
4. Add secondary antibody and incubate with gentle agitations over night at 4°C.
5. Wash the antibody X5 times with PBS, each wash for 1 hour with gentle agitations.

Mounting open book for imaging

1. Smear grease or silicone in a rectangle shape on a slide and drip PBS within.
2. Place the spinal cord stretched at the middle of the rectangle using tweezers and draw out the liquid around it with a Pasteur pipette.
3. Place 1-2 drops of mounting media on the spinal cord and cover with cover slip. Squash it to avoid air bubbles.
4. Keep the slides in dark in 4°C.
5. Inspect the spinal cord using confocal microscopy.

Discussion

Electroporation of plasmid DNA into the chick embryo evolves as a powerful technique for in vivo ectopic expression. The combination of specific enhancers, and chick electroporation provides a quick and efficient tool for deciphering axonal pathways of a genetically defined group of neurons [1,3,5]. Utilizing the Cre/LoxP and the Gal4/UAS amplification systems can augment the levels and duration of expression. The emerging picture is of a complex divergence of axonal cues that arises from interneuron subpopulations, defined by the direction of their axonal projections. In addition, simultaneous molecular and spatial restricted labeling of two neuronal populations can be attained. Thus, providing information about the architecture of neuronal circuits [3].

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